Molecular Ecology (1999) **8**, 1155–1170

Mitochondrial DNA variation in bull trout (*Salvelinus confluentus*) from northwestern North America: implications for zoogeography and conservation

ERIC B. TAYLOR,* SUSAN POLLARD† and DEREK LOUIE*
**Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 1Z4, Canada, †British Columbia Ministry of Lands, Parks, and Environment, Fisheries Branch, Conservation Section, 780 Blachard Street, Victoria, B.C. V8V 1X4, Canada*

Abstract

Bull trout, *Salvelinus confluentus* (Salmonidae), are distributed in northwestern North America from Nevada to Yukon Territory, largely in interior drainages. The species is of conservation concern owing to declines in abundance, particularly in southern portions of its range. To investigate phylogenetic structure within bull trout that might form the basis for the delineation of major conservation units, we conducted a mitochondrial DNA (mtDNA) survey in bull trout from throughout its range. Restriction fragment length polymorphism (RFLP) analysis of four segments of the mtDNA genome with 11 restriction enzymes resolved 21 composite haplotypes that differed by an average of 0.5% in sequence. One group of haplotypes predominated in 'coastal' areas (west of the coastal mountain ranges) while another predominated in 'interior' regions (east of the coastal mountains). The two putative lineages differed by 0.8% in sequence and were also resolved by sequencing a portion of the *ND1* gene in a representative of each RFLP haplotype. Significant variation existed within individual sample sites (12% of total variation) and among sites within major geographical regions (33%), but most variation (55%) was associated with differences between coastal and interior regions. We concluded that: (i) bull trout are subdivided into coastal and interior lineages; (ii) this subdivision reflects recent historical isolation in two refugia south of the Cordilleran ice sheet during the Pleistocene: the Chehalis and Columbia refugia; and (iii) most of the molecular variation resides at the interpopulation and inter-region levels. Conservation efforts, therefore, should focus on maintaining as many populations as possible across as many geographical regions as possible within both coastal and interior lineages.

Keywords: bull trout, mtDNA, postglacial dispersal, *Salvelinus*, zoogeography

Received 9 September 1998; revision accepted 9 February 1999

Introduction

Fishes of the genus *Salvelinus*, commonly known as 'char', have presented a great challenge to taxonomists and evolutionary biologists interested in resolving inter-relationships and understanding the evolution of diversity within salmonid genera (Behnke 1972; Nordeng 1983). The genus *Salvelinus* consists of three main lineages (up to 15 species) of largely freshwater salmonid fish native to the temperate and Arctic regions of the northern hemisphere (Behnke 1972, 1980). Many of the systematic uncertainties stem, in part, from the limitations of morphological analyses in a group of fish with extensive phenotypic plasticity. For example, Dolly Varden (*S. malma*) and bull trout (*S. confluentus*) are two morphologically very similar char with largely parapatric distributions in northwestern North America: Dolly Varden are found in coastal drainages from western Washington to western Alaska and bull trout are found largely in interior drainages from northern California to Yukon Territory (see fig. 1 of Baxter *et al*. (1997)). The two char are so similar morphologically and ecologically that at one time they were considered a single species, i.e. Dolly Varden (McPhail 1961; Morton 1970). More recent morphological analyses by Cavender

Correspondence: E. B. Taylor. Fax: 604-822 2416; E-mail: etaylor@zoology.ubc.ca

(1978) and Haas & McPhail (1991) suggested that the two char warrented distinct species status. This notion was supported by diverse molecular analyses that have demonstrated extensive molecular divergence between Dolly Varden and bull trout (Grewe *et al*. 1990; Pleyte *et al*. 1992; Crane *et al*. 1994). Subsequent genetic studies of the two char in sympatry have demonstrated that they are separate gene pools (Baxter *et al*. 1997; Leary & Allendorf 1997) providing the most compelling evidence that Dolly Varden and bull trout are distinct biological species.

As most evolutionary studies of bull trout have focused on their inter-relationships with other members of the genus, there have been no detailed studies of evolutionary divergence within the species over its extensive western North American range. Consequently, efforts to conserve evolutionary diversity within bull trout are proceeding without information on phylogenetic variation within the species. Indeed, conservation issues for bull trout have become increasingly important as the species has undergone significant declines in abundance and distribution, particularly in southern portions of its range (Mackay *et al*. 1997; Rieman *et al*. 1997), and two population segments have been declared endangered or threatened under the US Endangered Species Act (US Department of Interior Federal Register July 1998). Leary *et al*. (1993) surveyed allozyme polymorphism in bull trout from the upper Columbia and Klamath rivers while Williams *et al*. (1997) surveyed mitochondrial DNA (mtDNA) variation in the species over much the same geographical range. Although informative for bull trout within these large river systems, with the exception of one population, neither of these studies included populations from the largest portion of its range in Canada (i.e. British Columbia, Yukon, Northwest Territories, and Alberta) where populations are still relatively healthy. Morphological and meristic variation in bull trout was examined by Haas (1988) in specimens collected from throughout northwestern North America. His analysis suggested the existence of four major groups of bull trout. These morphological groupings were suggested to represent evolutionary lineages resulting from isolation in and subsequent dispersal from four Wisconsinan glacial refugia: the Chehalis, Pacific (lower Columbia), Missouri, and Bering/Nahanni (Haas 1988). If such morphological groupings indeed represent intraspecific phylogenetic divisions, they could form the basis for the recognition of primary conservation units below the species level (e.g. 'evolutionarily significant units' *sensu* Waples (1995)). Because, however, morphological distinctions may not represent phylogenetic divisions, but either phenotypic plasticity or adaptive variation (Hillis 1987; Taylor & Bentzen 1993), independent (from morphology) assessments of evolutionary diversity within bull trout from throughout its range are needed. The principle objective of our research was, therefore, to conduct a phylogenetic survey of bull trout from throughout northwestern North America. As the range of bull trout encompasses an area that includes several Wisconsinan glacial refugia, we tested the hypothesis that bull trout molecular variation could be partitioned across major lineages that stem from historical isolation of bull trout within these refugia. To assess this hypothesis, we surveyed bull trout for restriction site and sequence variation in the mtDNA genome, a procedure that has often proven very informative in resolving phylogenetic lineages within species (Avise 1994; Moritz 1994) including many fish groups (Bermingham & Avise 1986; Bernatchez & Wilson 1998; Redenbach & Taylor 1999).

Materials and methods

Sampling strategy and sample sites

Bull trout were sampled from a total of 47 populations from throughout its range (Fig. 1). Samples consisted

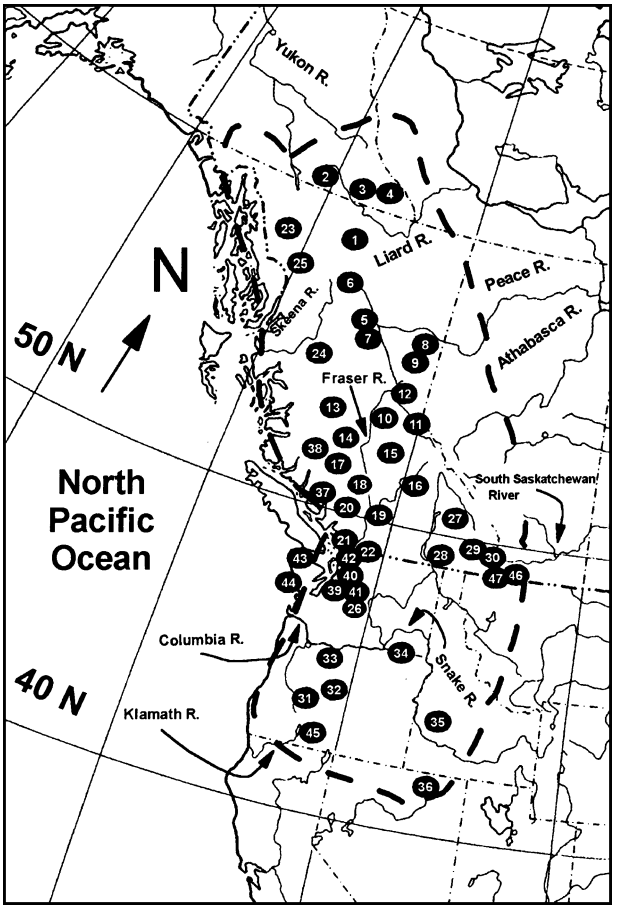

Fig. 1 Distribution of populations of bull trout sampled for mitochondrial DNA (mtDNA) analysis. Sample codes are identified in Table 1. The dashed line encompasses the approximate natural range of bull trout.

largely of fin clips stored in 95% ethanol, but some frozen tissues were also used. We were interested in broad-scale geographical patterns of variation and previous studies have shown that biochemical or molecular variation within populations is minimal (Leary *et al*. 1993; Williams *et al*. 1997). Therefore, we focused on sampling as many sites as possible, but limited our sampling within sites to usually five to 10 individuals. Owing to their remoteness, some of our sites could not be sampled extensively and consisted of samples of less than five individuals. Our geographical coverage included all areas now occupied by bull trout that may have either served as Pleistocene glacial refugia themselves or that have been postglacially colonized from the major refugia (Beringia, lower Columbia River, upper Missouri, Chehalis, and Nahanni River; Table 1). We also included three Dolly Varden (*Salvelinus malma*) and a single brook trout (*S. fontinalis*) as outgroup taxa in our analyses. The Dolly Varden were collected from the Keogh River on northeastern Vancouver Island and from the Iskut River in north coastal British Columbia, while the brook trout was sampled from a naturalized population in the Beaver River, a tributary of the upper Columbia River near Trail, British Columbia.

Molecular analyses: restriction fragment length polymorphisms (RFLPs)

We surveyed mtDNA variation by examining restriction site variation in four amplified portions of the mtDNA genome in all samples as well as by sequence analysis of a fifth region. We used the polymerase chain reaction (PCR) to amplify a 2.1 kb fragment consisting of cytochrome *b* and the control region and a 2.5 kb fragment consisting of the NADH 5 and 6 (*ND5/6*) genes. Restriction or sequence analysis of targeted regions of mtDNA to resolve major lineages is an efficient alternative to whole molecule analysis (e.g. O'Reilly *et al*. 1993; Ortí *et al*. 1994). The cytochrome *b*/control region was amplified using the primers HN20 (Bernatchez & Osinov 1995) and the reverse complement of C-Glu (Park *et al*. 1993), while the *ND5/6* genes were amplified using C-Glu and C-Leu3 (Park *et al*. 1993). The PCR conditions were as described in Redenbach & Taylor (1999). The PCR-amplified segments were pooled post-PCR and digested with 11 restriction enzymes: *Alu*I, *Ava*II, *Bst*UI, *Dpn*II, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Nla*III, *Rsa*I, and *Taq*I under conditions outlined by the manufacturer (NEB). DNA restrictions were resolved on 2.0% agarose gels, stained with ethidium bromide, and photographed under ultraviolet (UV) light.

Molecular analyses: sequencing

We sequenced a portion of the *ND1* gene of the mitochondrial genome in all haplotypes defined by RFLP analysis (see below). We examined this gene to obtain coverage of nucleotide variation in regions distinct from our RFLP survey and because it was shown by Williams *et al*. (1997) to be polymorphic in bull trout. We used the primers t-Ile (Park *et al*. 1993) and ND1C (Redenbach & Taylor 1999) to amplify ≈ 1.6 kb of mtDNA encompassing the tRNA-Ile and a portion of the *ND1* gene. The t-Ile primer was then used to sequence ≈ 550 bp from the 3′ end of the *ND1* gene. The procedures for obtaining the *ND1* sequence via automated sequencing were as described by Redenbach & Taylor (1999).

Data analyses

All RFLPs in bull trout could be accounted for by single or double restriction site changes. Consequently, a presence/absence restriction site matrix was contructed for each RFLP observed for each enzyme and was given a single capital letter code (e.g. *Alu*I A, B, C, etc.). Each fish was then characterized by an 11 letter composite haplotype code; each letter representing the restriction site code for each of the 11 enzymes (e.g. AAAAAAAAAAA, ABAAAAAAAAA, etc.). A site matrix for each of the composite haplotypes resolved was constructed using the program REAP (McElroy *et al*. 1992). The composite haplotype restriction site matrix so generated formed the basis for subsequent analyses of haplotype and nucleotide diversity using programs in REAP.

Divergence among haplotypes was estimated as the number of nucleotide substitutions per site, *d* (Nei & Miller 1990). Patterns of similarity among haplotypes were summarized by using the unweighted pair group method with arithmetic averages (UPGMA; Sneath & Sokal 1973) to cluster sequence divergence estimates using NEIGHBOUR of PHYLIP (version 3.5; Felsenstein 1993). Divergence among aligned *ND1* sequences were examined using Kimura's 2-parameter distance (using DNADIST of PHYLIP) because transition subsitutions greatly outnumbered transversions. Relationships among sequence haplotypes were estimated by Wagner parsimony using DNAPARS of PHYLIP. Finally, we combined the RFLP and sequence information into a single data matrix by converting the sequence information into binary (1,0) format. The resulting 615 character matrix was analysed by Wagner parsimony using MIX of PHYLIP accompanied by 1000 bootstrap replicate analyses of the matrix. Total nucleotide diversity was partitioned into variance components by using the analysis of molecular variance (AMOVA) approach of Excoffier *et al*. (1992). Both haplotype frequency and level of evolutionary divergence among haplotypes were utilized in extracting variance components, although the results were very similar if only frequency differences among localities were used. Initially we pooled sites into 'coastal' (at or west of the

Table 1 Sample populations and number code (see Fig. 1), sample sizes, haplotype diversity (with standard error (SE)) and nucleotide diversity (× 100) of bull trout collected in the study. Diversity statistics are presented for those samples with *n* of at least five individuals

Population (no.)	Sample size	Haplotype diversity	Nucleotide diversity	Population (no.)	Sample size	Haplotype diversity	Nucleotide diversity
Upper Liard River				Upper Columbia River			
Hotel Cr. (1)	12	0.0000	0.0000	Yakima R. (26)	9	0.5490 (0.1264)	0.2130
Shilsky L. (2)	4	—	—	Duncan L. (27)	8	0.0000	0.0000
Lower Liard River				Salmo R. (28)	13	0.0000	0.0000
Beaver R. (3)	5	0.0000	0.0000	Wigwam R. (29)	11	0.3117 (0.1065)	0.0409
Crow R. (4)	5	0.0000	0.0000	Howell Cr. (30)	8	0.0000	0.0000
Upper Peace River				Lower Columbia R.			
Mesilinka R. (5)	8	0.5667 (0.1089)	0.1794	Anderson Cr. (31)	5	0.0000	0.0000
Chowika R. (6)	5	0.3556 (0.1591)	0.0501	Metolius R. (32)	13	0.0000	0.0000
Osilinka R. (7)	6	0.0000	0.0000	Clear Branch Cr. (33)	3	—	—
Lower Peace River				Snake R.			
Burnt R. (8)	6	0.4848 (0.1059)	0.0656	Bear Cr. (34)	5	0.0000	0.0000
Belcourt L. (9)	8	0.0000	0.0000	Boise R. (35)	3	—	—
Upper Fraser River				Jarbridge R. (36)	10	0.0000	0.0000
Dome Cr. (10)	10	0.5895 (0.0926)	0.2665	South Coast/Puget Sound			
Small Cr. (11)	7	0.5275 (0.0636)	0.2876	Squamish R. (37)	10	0.1895 (0.1081)	0.1007
Upper Torpy R. (12)	7	0.0000	0.0000	Klinaklini R. (38)	10	0.4421 (0.0876)	0.0574
Middle Fraser River				Puyallup R. (39)	1	—	—
Chilko R. (13)	5	0.5333 (0.0947)	0.0737	White R. (40)	1	—	—
Grain Cr. (14)	5	0.0000	0.0000	Mud Mtn. Dam (41)	2	—	—
North Thompson R. (15)	10	0.7582 (0.0504)	0.3632	Skagit R. (42)	7	0.5275 (0.0638)	0.0014
Shuswap R. (16)	5	0.0000	0.0000	Elwha R. (43)	14	0.0000	0.0000
Nahatlach R. (17)	5	0.5333 (0.0947)	0.3723	Soleduck R. (44)	10	0.0000	0.0000
Dominion Cr. (18)	5	0.3556 (0.1591)	0.2482	Klamath R.			
Upper Anderson R. (19)	6	0.4848 (0.1059)	0.2003	Long Cr. (45)	5	0.0000	0.0000
Lower Fraser River				South Saskatchewan R.			
Upper Pitt R. (20)	19	0.3414 (0.0776)	0.2177	Belly R. (46)	5	0.0000	0.0000
Chillwack L. (21)	12	0.0000	0.0000	Waterton R. (47)	5	0.0000	0.0000
Silverhope Cr. (22)	2	—	—				
North Coast							
Tahltan R. (23)	5	0.0000	0.0000				
Bulkley R. (24)	8	0.5333 (0.0456)	0.1403				
Nass R. (25)	13	0.0000	0.0000	Average	Total *n* = 348	0.1800 (0.0014)	0.0709 (0.0003)

Cascade/Coast mountain ranges) and 'interior' (east of these mountains) regions as suggested by clustering and parsimony analyses of the RFLP and sequence haplotype character matrices (see below) and because this grouping has been recognized in previous studies of fish (e.g. Parkinson 1984; Taylor & McPhail 1985a; Haas 1988). We also tested alternative hierarchies including pooling sites into 'northern' (all river systems north of the Fraser River) and 'southern' (the Fraser River and all systems to the south) regions because this geographical split has also been resolved in northwestern North American fishes (Utter *et al*. 1980; Taylor 1995; Taylor *et al*. 1996; Thompson *et al*. 1997). Sample localities were also pooled into 12 different watershed regions (upper Liard, lower Liard, upper Peace, lower Peace, North Coast, upper Fraser, middle Fraser, lower Fraser and south coast British Columbia, Puget Sound, upper Columbia, lower Columbia/California, South Saskatchewan). These same watershed groups were analysed by AMOVA in a separate analysis, this time examining watersheds within each of the coastal and interior regions. Finally, we pooled sites into groups of populations that are thought to have originated from postglacial colonization from four major refugia: Bering/Nahanni, Chehalis, Missouri/Great Plains, and lower Columbia River Valley (McPhail & Lindsey 1986). The AMOVA analyses were summarized by the calculation of variance components and their associated ϕ statistics, i.e. among major regional groupings (e.g. ϕ_{CT}), followed by variation among sample sites within each of these regional groupings (ϕ_{SC}) and variation within individual sites (ϕ_{ST}). The statistical significance of each of the variance components was estimated by random permutations of the original restriction site and haplotype frequency matrices (Excoffier *et al*. 1992). In the AMOVA analyses, we only used sites with samples sizes of at least five individuals (n = 40 sites).

Results

RFLP haplotype diversity and inter-relationships

Our analysis surveyed 115 restriction sites over 410 bp. Thirty-three sites were variable and 21 bull trout haplotypes were resolved among the 348 samples examined (Table 2). Average pairwise sequence divergence among haplotypes was 0.54% (standard error (SE) = 0.02) and ranged from 0.13 to 1.2%. Dolly Varden mtDNA (n = 3 from two different localities) differed from bull trout at seven of the 11 restriction enzymes and averaged 1.8% (SE = 0.1, range 1.3–2.4%) divergent from bull trout haplotypes.

Clustering of the bull trout haplotype sequence divergence estimates resolved two broad groupings of haplotypes: group A consisted of haplotypes 8–12; group B consisted of all other haplotypes (1–7, 13–14, 15–21; Fig. 2) and these two groups differed by about 0.8% in sequence. Most of the values for pairwise sequence divergences between all haplotype pairs that were above the median value (0.53% divergence) involved comparisons between haplotypes from these mtDNA groupings A and B (76%) rather than between haplotypes within either of these groups (24%) and the distribution of haplotypes when subdivided into groups A and B was highly structured geographically (see below).

These same two groupings were recovered in neighbour-joining analysis of the sequence divergence estimates as well as in Wagner parsimony analysis of the presence/absence restriction site matrix (dendrograms not shown). Statistical support for a distinction between these two putative groupings using UPGMA, neighbour-joining or parsimony analyses, however, was weak. The node separating groups A and B was found in only a maximum of 52% of the bootstrap replicates and they differed by only a single diagnostic site resolved with *Nla*III. The inclusion of Dolly Varden as an outgroup in these analyses compromised the integrity of group A haplotypes especially. Haplotypes 11 and 12 typically formed an

Table 2 Composite haplotypes of bull trout mitochondrial DNA (mtDNA) resolved with 11 enzymes and their approximate mean percentage frequency across all samples. Haplotype 22 represents Dolly Varden. Each letter represents the restriction fragment length polymorphism resolved with *Alu*I, *Ava*II, *Bst*UI, *Hha*I, *Hae*III, *Hin*cII, *Hin*fI, *Nla*III, *Rsa*I, *Sau*3AI, and *Taq*I, respectively

Haplotype number	Haplotype code	Mean percentage occurrence
1	AAAAAAAAAAA	47.0
2	AAACAAAAAAA	1.9
3	AAAAAABAAAA	1.5
4	BBAAAAAABAA	1.8
5	AABBAAAAAAA	4.7
6	AAAAAAAAAAB	4.5
7	AAAAAAAABAA	2.3
8	CAAABABBBAA	2.3
9	CCAAAABBCAA	5.0
10	CCAAAABBBAA	7.4
11	CAAAAACCBAA	8.2
12	CAAACACCBAA	0.2
13	CAAABAAAAAA	7.8
14	CAAAAAAAAAA	2.6
15	CAAAAABBAAA	2.3
16	AAAAAABEAAA	2.3
17	CBAAAAAABAA	0.5
18	CAAAAABAAAA	0.3
19	CAAABABAAAA	1.0
20	AAAAAADAAAA	0.4
21	CAAAAAAAAAD	2.3
22	DABDCBBDDAC	—

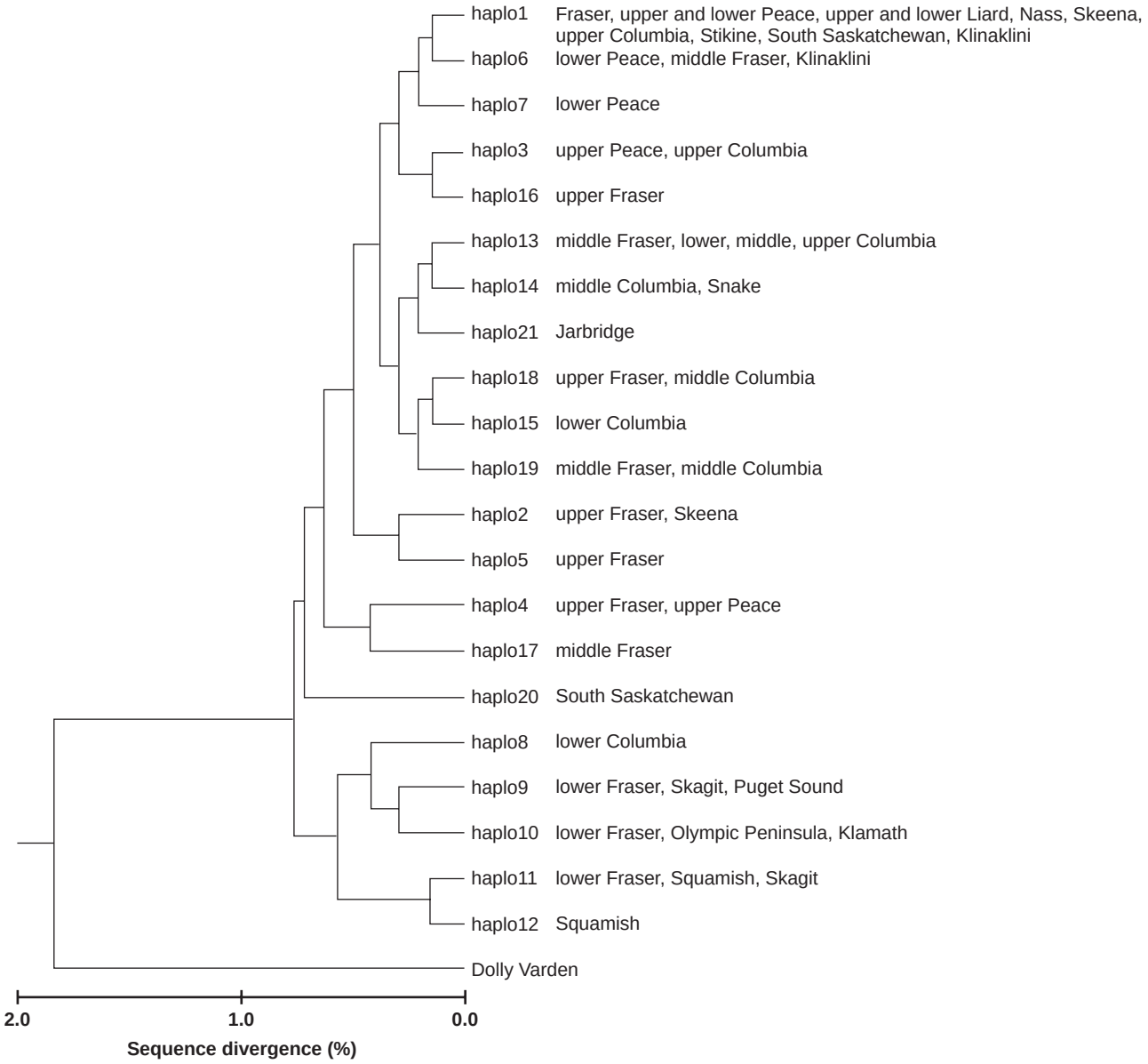

Fig. 2 UPGMA dendrogram of pairwise sequence divergence estimates among 21 bull trout restriction fragment length polymorphism (RFLP) haplotypes with a Dolly Varden haplotype for comparison. Also shown are the geographical locations in which each haplotype occurred at least once.

unresolved trichotomy with Dolly Varden mtDNA and the remaining bull trout haplotypes, using neighbour-joining or parsimony analyses.

Sequence analysis

To increase the resolution of the bull trout haplotype trees, we sequenced a representative of each RFLP haplotype (including the Dolly Varden haplotype). We also included a brook trout in our sequencing owing to the ambiguity introduced into our phylogenetic analyses by using Dolly Varden mtDNA as an outgroup. Brook trout have been clearly resolved from both bull trout and Dolly Varden using mtDNA (Grewe *et al*. 1990).

We obtained 500 bp of sequence from the 3′ end of the *ND1* gene in the 21 bull trout RFLP haplotypes and in one Dolly Varden and one brook trout (Fig. 3). In total, however, we identified only six different sequence haplotypes in bull trout because RFLP haplotypes 1–5, 13, 16, 18–21 all had identical *ND1* sequences as did haplotypes 11–12 and haplotypes 9, 10, and 15. Thirty-eight sites were variable with 29 of these distinguishing all bull trout from

```
                    1 2 4 4 6 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3
                    5 3 2 4 6 2 4 5 6 7 1 2 4 6 6 7 7 8 9 0 0 1 2 4 5 5 5
                              0 3 1 8 3 2 8 0 1 7 0 9 5 1 0 5 4 6 2 4 6 9

Haplotype 1–3,      G T A T C A G G C C A G T C C A T A T A T G C G G C C
7,13,16,18–21
Haplotype 14        . . . . . T . . . . . . . . . . . . . . . . . . . . .
Haplotype 17        . . . . . G . T . . . . . . . . . . . . . . . . . . .
Haplotype 8         . . . . . . . A T . . . . . . . . . C . . . . . . . T
Haplotype 9,10      . . . . . . . G T . . . . . . . . . C . . . . . . . C
Haplotype 11,12     . . . . T . . . T . . . . . . . . . C . . . . . . . T
Haplotype 15        . . . . C . . . T . . . . . . . . . C . . . . . . . C
Dolly Varden        . . . . . . . . T . . . . . . . . . C . C A . . . . C
Brook Trout         T G G A T G G G T T G A G T T G A G A G T G T T A T T
                                                        *

                    3 3 4 4 4 4 4 4 4 4 4
                    6 7 4 4 4 5 6 6 7 7 9
                    6 7 0 3 9 4 1 4 0 5 9

Haplotype 1–7,      T G A T A A T T T G A
13,16–21
Haplotype 14        . . . . . .
Haplotype 17        . . . . . .
Haplotype 8         . . . . . . . C . .
Haplotype 9–10      . . . . . . . C . .
Haplotype 11,12     . . . . G . . . C . .
Haplotype 15        . . . . A . . . C . .
Dolly Varden        . . G . A . . . T A G
Brook trout         G A G C G G C C T A A
                                  *
```

Fig. 3 Variable positions for a 500 bp sequence encompassing part of the 16S rRNA gene (bp 1–199), Leu-tRNA (bp 200–275, and NADH-1 gene (bp 276–500). Sequences for bull trout are listed according to restriction fragment length polymorphism (RFLP) haplotypes defined in Table 2 (see also Fig. 2) plus representative brook trout and Dolly Varden sequences. Identical positions are indicated by a '.', while asterisks indicate diagnostic positions for 'coastal' and 'interior' groups of bull trout. Sequences have been deposited in GenBank under accession nos AF126000–AF126007.

brook trout and five sites distinguishing Dolly Varden from bull trout. Of the remaining variable positions, two marked changes (C ↔ T transitions) that distinguished the five group A RFLP haplotypes from the 15 group B RFLP haplotypes (Fig. 3). Bull trout *ND1* averaged 1.5% (SE = 0.13) divergence from Dolly Varden and 7.1% (SE = 0.11) divergence from brook trout, while Dolly Varden and brook trout sequences also differed by 7.1%. Variation among bull trout haplotypes averaged 0.8% (SE = 0.11).

When clustered by UPGMA, the sequence divergence estimates among *ND1* haplotypes also fell into two groups corresponding to RFLP haplotype groups A and B (dendrogram not shown). Haplotypes within these two groups differed by an average of 1.01% (SE = 0.13) in sequence, a value slightly greater than that based on our RFLP analysis (0.8%). Variation within the coastal and interior sequence haplotypes averaged 0.53% (SE = 0.06) and 0.30% (SE = 0.07), respectively. Wagner parsimony analysis of the pooled RFLP and sequence information produced a similar topology to that of the RFLP (or sequence) data analysed alone, but with higher levels of bootstrap support (Fig. 4). A monophyletic assemblage of haplotypes (82% support) was identified that consisted of haplotypes previously designated as group B by RFLP analysis. A second set of haplotypes (8–12, 15), although clearly distinct from group B haplotypes, was usually not monophyletic, but rather formed a grade between outgroup taxa and group B bull trout haplotypes in the

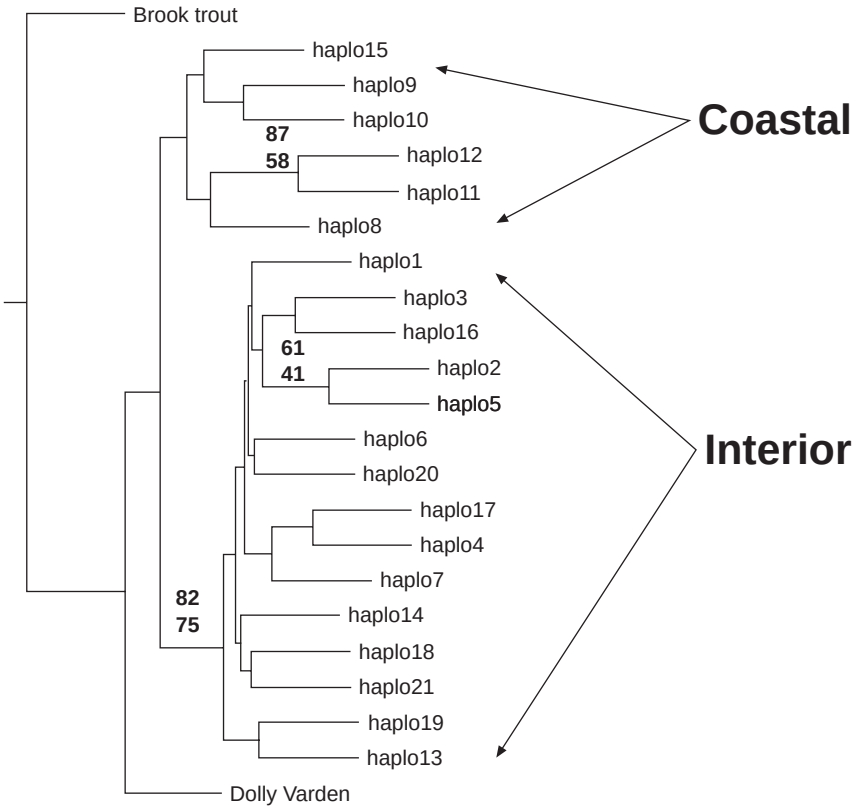

Fig. 4 Consensus tree of parsimony analysis of relationships among bull trout, Dolly Varden, and brook trout (root) restriction fragment length polymorphism (RFLP) and NADH-1 sequence haplotypes. The tree was derived from a composite matrix of RFLP restriction sites as well as sequence variants transformed to 1,0 format. Bull trout haplotypes are RFLP haplotypes defined in Table 2. The numbers at branch points represent the percentage of 1000 bootstrap replicates containing the group to the left of each branch point (numbers above: combined RFLP/sequence analysis; numbers below: sequence analysis only).

majority of bootstrap replicates (Fig. 4). The only other strongly supported group of haplotypes was that consisting of numbers 11 and 12 which was found in 87% of the maximum parsimony analyses.

Geographical distribution of mtDNA haplotypes

The distribution of bull trout mtDNA haplotypes was highly structured geographically (Figs 2, 5). Almost without exception, group A haplotypes were found in drainages at or west of the Coast or Cascade mountain ranges that separate much of northwestern North America into distinct 'coastal' and 'interior' ecoregions. Group A haplotypes were found in southwestern British Columbia in the lower Fraser River (below the Hell's Gate canyon area) and adjacent coastal streams (e.g. Squamish (population number 37) and Skagit (42) rivers), the Olympic Peninsula and Puget Sound (39–44), the lower Columbia River (below the Snake River), and in the Klamath River (45) in southwestern Oregon. By contrast, group B haplotypes were exceptionally widespread in northcentral and northwestern British Columbia and southern Yukon (e.g. Stikine, upper Yukon, and Liard rivers), the Peace, upper Fraser, upper Columbia/Kootenay and Snake rivers, and in the South Saskatchewan drainage east of the continental divide (Figs 2, 5). The only exceptions to this general pattern were the presence of group B haplotype 6 in three fish from the Klinaklini River (38, British Columbia south coast) and of group B haplotype 13 in Clear Branch Creek (33, lower Columbia River drainage; Figs 2, 5). The transition between coastal and interior bull trout mtDNA appears to be abrupt, at least in the Fraser River. Less than 30 km of river separates the most upstream population with coastal haplotypes (Silverhope Creek, 22) from the most downstream population with interior haplotypes (Upper Anderson River, 19; Fig. 5). The intervening area was sampled extensively, but no other populations of bull trout were located. The transition between coastal and interior haplotypes appears less abrupt in the lower Columbia River. Interior and coastal haplotypes were mixed in the area of the 'Columbia Gorge'; the most downstream interior group of haplotypes was located in Clear Branch Creek (33) while samples from the upper Metolius River (32) at the eastern edge of the Cascade Crest contained coastal haplotypes (Fig. 5).

Hierarchical analysis of mtDNA diversity

The partitioning of the RFLP nucleotide variation was examined by pooling samples site data in several different hierarchical arrangements (Table 3). Pooling sites into 'coastal' and 'interior' regions, as suggested by clustering and parsimony analyses of the RFLP and sequence haplotype character matrices (Figs 2, 5), resulted in the highest

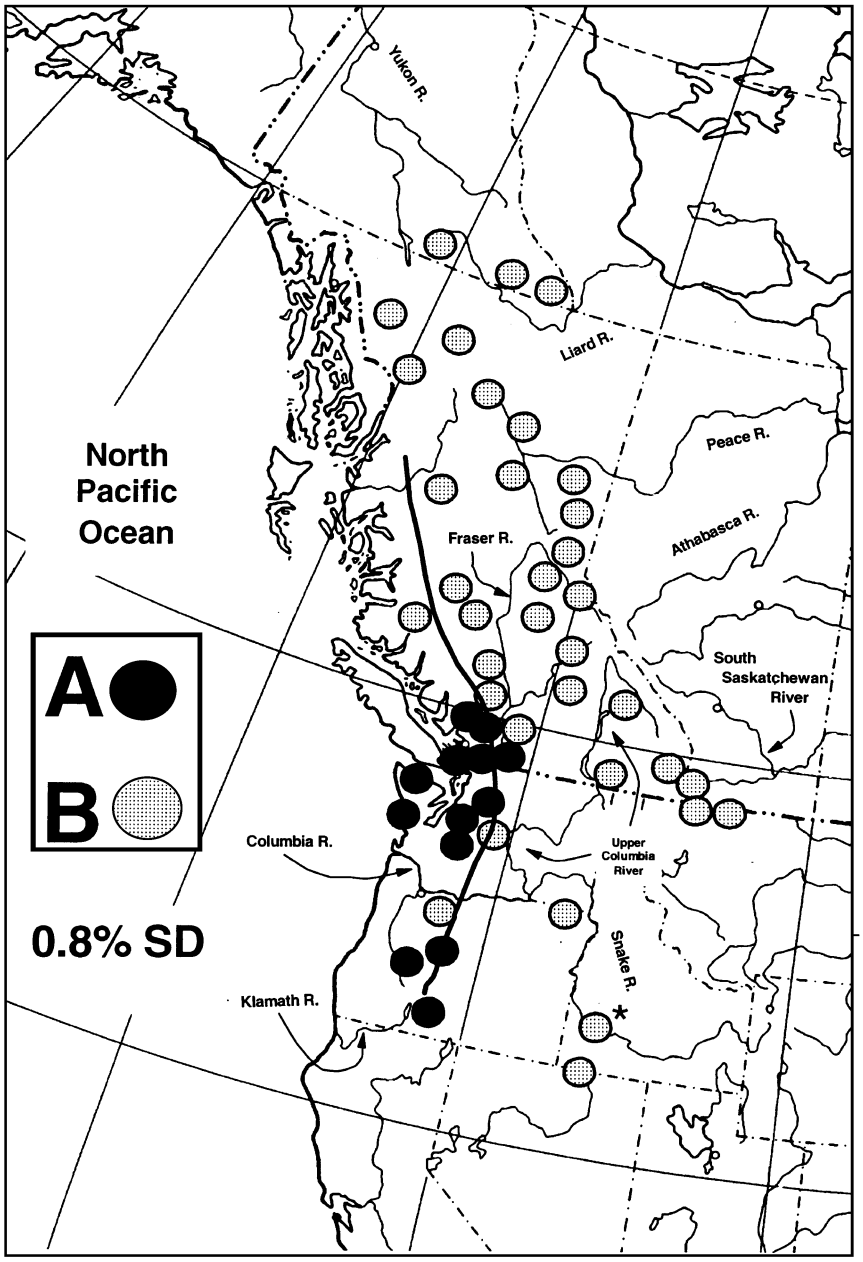

Fig. 5 Distribution of two major bull trout mitochondrial DNA (mtDNA) groupings identified by restriction fragment length polymorphism (RFLP) and sequence analyses. Group A = haplotypes 8–12, 15 and group B = haplotypes 1–7, 13–14, 16–21 (see Figs 2,4). The solid black line dividing groups A and B represents the approximate location of the Cascade/Coast Mountain crest. The Boise Creek population (*) was assayed by sequence analysis.

degree of variation explained at the regional level (55%; Table 3). Pooling sites by putative refugia also resolved substantial among-group variability (47%), but the high intergroup variability appeared to be almost entirely due to the differences between populations at or west of the Cascade/Coast mountain range and all other areas. When the interior sites were examined by pooling into three putative refuge groups (Bering/Nahanni, Missouri/Great Plains, upper Columbia), the variance component attributable to different refugia declined to 1.5% ($P = 0.34$; Table 3). Although the majority of variation resided at the level of large-scale geographical regions, no matter which hierarchical arrangement was tested there was always significant variation detected among sample sites within larger geographical regions (Table 3).

Haplotype diversity within populations

Bull trout appear to be characterized by having relatively little mtDNA variation resident within populations. In few of the AMOVA analyses cited above was the component of within-population variation greater than 20% of the total nucleotide variation, although it was always statistically significant. Similarily, for those sites where

Table 3 Hierarchical analysis of mitochondrial DNA (mtDNA) diversity in bull trout from AMOVA (see text for details)

Geographical arrangement	Variance component	ϕ statistic	Percentage of variation	*P*
Coastal vs. interior	Among regions	0.5529	55.2	0.0000
	Among sites	0.7359	32.7	0.0000
	Within sites	0.8805	11.9	0.0000
North vs. south	Among regions	0.2549	25.5	0.0021
	Among sites	0.8081	60.2	0.0000
	Within sites	0.8570	14.3	0.0000
Major watersheds	Among regions	0.4355	43.6	0.0000
	Among sites	0.7125	40.2	0.0000
	Within sites	0.8377	16.2	0.0000
Coastal region	Among watersheds	0.1979	19.7	0.0675
	Among sites	0.8004	64.2	0.0000
	Within sites	0.8399	16.0	0.0000
Interior region	Among watersheds	−0.0077	−0.77	0.5810
	Among sites	0.6334	63.9	0.0000
	Within sites	0.6309	36.9	0.0000
Refugia	Among refugia	0.4707	47.1	0.0000
	Among sites	0.7381	39.1	0.0000
	Within sites	0.8651	13.1	0.0000
Interior refugia	Among refugia	0.0151	1.5	0.3470
	Among sites	0.6288	61.4	0.0000
	Within sites	0.6289	37.1	0.0000

sample sizes were at least 10 (n = 14 populations), haplotype diversity averaged 0.21 (SE = 0.07, range = 0.0–0.59; Table 1). Nucleotide diversity (× 100) was similarly low, averaging 0.08 (SE = 0.03, range = 0.0–0.32; Table 1). Over the entire geographical range, however, bull trout mtDNA was highly variable; haplotype diversity was 0.8383 (SE = 0.0399) and nucleotide diversity was 0.003418 when we pooled haplotypes across all localities.

Discussion

Diversity and inter-relationships of bull trout mtDNA

The depth of divergence among bull trout mtDNA haplotypes was relatively shallow (average pairwise divergence of 0.5%, maximum of 1.2%) and there was a subdivision of haplotypes into two geographical groupings: 'coastal' and 'interior' (Figs 2, 5). The degree of evolutionary divergence among bull trout haplotypes is similar to that in Holarctic fishes summarized by Bernatchez & Wilson (1998). The shallow phylogenetic depth of mtDNA in these fishes (average maximum d of ≈ 1.2% from 25 species; Bernatchez & Wilson 1998), relative to fishes from more southern localities in North America and Europe, is thought to stem from the greater reduction of genetic diversity in northern fishes through extinction of divergent lineages found in habitats affected more directly by Pleistocene glacial events (e.g. Bernatchez & Wilson 1998). Within this context, the phylogeographical structure of bull trout mtDNA is subtle compared with deeper divisions observed in other northwestern North American fishes. For instance, both three-spine sticklebacks (*Gasterosteus aculeatus*) and rainbow trout (*Oncorhynchus mykiss*) exhibit phylogenetic divisions of mtDNA into major clades that differ in sequence from about 2.5% (O'Reilly *et al*. 1993; Thompson *et al*. 1997) to 1.8% (M. McCusker & E. B. Taylor, unpublished), respectively. The relative shallowness of the bull trout phylogenetic division may reflect: (i) more recent isolation of bull trout in refugia during late Pleistocene glacial events; (ii) a lower mutation rate in bull trout mtDNA; or (iii) lower evolutionarily effective population sizes in bull trout relative to these other species.

All 'coastal' haplotypes (8–12, 15) were closer to the root of the parsimony tree than were the more derived 'interior' haplotypes (13–14, 16–21). Further, sequence sites that characterized the coastal haplotypes were usually shared with either Dolly Varden or brook trout or both of these outgroup taxa (Fig. 3). These data suggest that the coastally distributed bull trout haplotypes are decendants of lineages that more closely resemble ancestral bull trout mtDNA (i.e. near the split between bull trout and other western *Salvelinus*).

The apparent greater antiquity of coastal haplotypes may stem from a higher probability of persistence of older haplotypes in coastal areas owing to their proximity to presumed Pleistocene refugia for bull trout (see below). By contrast, most of the geographical areas where interior haplotypes were found were repeatedly exposed to more complete destruction by glacial advances and retreats over the 20 or so Pleistocene glacial events (Lindsey &

McPhail 1986; McPhail & Lindsey 1986; Martinson *et al*. 1987). It is possible, therefore, that interior haplotypes were subject to greater probability of extinction for older and intermediate haplotypes than coastal haplotypes resulting in the predominance of more highly derived haplotypes in interior regions. This conjecture is supported by: (i) a higher (2.1 vs. 1.5) average number of pairwise site differences between coastal haplotypes relative to comparisons between interior haplotypes; (ii) a higher (0.72 vs. 0.61) haplotype diversity within the coastal region (pooled across localities); and (iii) the observation that the most basal interior haplotypes are found in areas that were probably closest to presumed lower Columbia Pleistocene refugia (e.g. haplotypes 18 and 19 in the Yakima River (26), haplotype 13 in the Snake River (34), haplotype 13 in the lower Columbia River (33); (Figs 2, 5).

Geographical distribution of mtDNA haplotypes

Although the depth of the bull trout mtDNA haplotype separation was shallow, there was a sharp discontinuity in the geographical distribution of group A and B haplotypes (Fig. 2). Most group A are 'coastal' (at or west of the Coast and Cascade mountain crests), while all but two group B haplotypes were found east of these mountain crests. The only exceptions to this pattern involved two haplotypes (6 and 13) that, although they were typically found in interior localities, were also recorded in two coastal-draining streams (Figs 2, 5). Discontinuity in the geographical distribution of haplotypes suggests that there has been a strong historical component to the organization of bull trout mtDNA diversity. A probable source of historical effects is isolation during the late Pleistocene in distinct glacial refugia (McPhail & Lindsey 1986; Wilson *et al*. 1996).

Our results, however, did not find multiple divergent mtDNA haplotypes associated with some geographical locations proposed to have acted as Wisconsinan glacial refugia based on morphological analyses (Haas 1988), e.g. Beringia (Liard River), Nahanni (Liard, Peace system), or upper Missouri River (South Saskatchewan River, upper Columbia and Kootenay rivers). Instead, most fish east of the coastal mountain ranges were characterized by mtDNA group B with little structure apparent among watersheds within that group (Table 3). Group A bull trout were clustered around the south coastal region of British Columbia, Puget Sound and the Olympic Peninsula in western Washington, the lower Columbia River, and the Klamath River in southwestern Oregon. These latter areas include the so-called 'Chehalis Refuge', a region dominated by drainages of the Chehalis River between the Columbia River and Puget Sound that is known to have been ice free during much of the Pleistocene. The Chehalis River valley has been suggested as a west coast refuge that was independent from the Columbia Refuge based on the distribution of endemic species and differentiated populations in fishes and plants (McPhail 1967; McPhail & Lindsey 1986; Haas 1988; Buckingham *et al*. 1995; Soltis *et al*. 1997; McPhail & Taylor 1999). The localization of group A haplotypes in this area suggests that the Chehalis Valley served as a Pleistocene refuge for group A bull trout haplotypes. Postglacial dispersal of coastal bull trout into the lower Fraser or Columbia rivers or adjacent coastal systems (e.g. Squamish River, Puget Sound rivers) from a Chehalis refuge may have occurred via freshwater connections through the Puget lowlands (McPhail 1967; Thorson 1980), or perhaps via the sea (Cavender 1997). Coastal bull trout have been observed to enter and successfully disperse in near shore marine areas (Cavender 1978; Haas & McPhail 1991).

The other potential refuge for bull trout during much of the Pleistocene was the Columbia River (including the Snake River) south of the ice sheet. This area probably served as the source of postglacial colonists for bull trout throughout the interior regions of the upper Columbia in the US and Canada and right through to more northern and eastern draining systems (i.e. Liard River in British Columbia, lower Peace, Athabasca, and South Saskatchewan rivers) via well-documented postglacial connections among these river systems (Lindsey & McPhail 1986; McPhail & Lindsey 1986; Haas 1988; Cavender 1997). Notwithstanding the presumed role of the lower Columbia River valley as a second glacial refuge for bull trout, the observation that group A haplotypes predominate in the Columbia area at or west of the Cascade Crest suggests that this region of the Columbia may have been largely colonized from the Chehalis Refuge. Fish surviving glaciation in the lower Columbia refuge may, therefore, have been concentrated east of the Cascade Crest and dispersed mostly inland into the upper Columbia, Fraser and other northern interior drainages. The idea that the 'lower Columbia' (below the confluence with the Snake River) may not be a single faunal unit in terms of postglacial dispersal of freshwater fish has been suggested previously as several other species that are widespread in the Columbia (and clearly had a Columbia origin postglacially) are curiously absent from the lower river (McPhail & Lindsey 1986).

An abrupt transition between populations bearing coastal haplotypes and those bearing interior haplotypes in the Fraser River (Fig. 5) is not surprising. The transition point encompasses the Fraser Canyon, a well-known area of difficult fish passage. The Fraser Canyon also marks a region of biogeoclimatic change from coastal wetlands to dry interior and is associated with abrupt shifts in the distribution of genetic variation within some fish species (e.g. Parkinson 1984; Taylor & McPhail 1985a,b; Wehrhahn & Powell 1987; Wood *et al*. 1994) as

well as with changes in the geographical distribution of several fishes (McPhail & Lindsey 1986). The 'Columbia Gorge' is where the lower Columbia breaks through the Cascade Range and includes the area between the Metolius River (a tributary of the Deschutes River) and Clear Branch Creek (a tributary of the Hood River). This area contained fast water chutes, at least during historic times, but does not appear to have been as much of an impediment to bull trout movement as the Fraser Canyon has been on the Fraser because coastal and interior haplotypes both were found in this portion of the Columbia.

The other site that appeared to have anomolous mtDNA haplotypes given its geographical location was the Klinaklini River (38). These char had haplotypes 1 and 6, both of which were typically found in interior areas. Interior bull trout haplotypes may, therefore, be found in other coastal systems in low frequency with further sampling. Alternatively, headwater faunal exchanges could explain the presence of interior haplotypes in coastal streams. The headwaters of the Klinaklini system are immediately adjacent to headwaters of the Chilcotin River system of the interior plateau. Both headwater systems are within a few kilometres of one another and may well have participated in faunal exchanges during deglaciation as has been suspected for other areas along the coastal–interior divide (McPhail & Lindsey 1986; Baxter et al. 1997). The sharing of the *Taq*I 'B' haplotype between some fish in the Klinaklini and in the Chilko River (13, a major Chilcotin tributary; Table 4) is consistent with this hypothesis. Headwater faunal exchanges may also explain why all large coastal-draining systems north of the Squamish River (e.g. Skeena, Stikine, Nass) had 'interior' haplotypes. All of our samples (including nine of the 10 Klinaklini samples) came from upstream portions of these systems and these rivers have extensive headwater tributaries that interdigitate with one another or with interior drainages of the Fraser and MacKenzie rivers (via the Peace and Liard rivers) among which faunal exchanges have taken place in the past (Lindsey & McPhail 1986; McPhail & Lindsey 1986). Therefore, bull trout probably colonized these systems by watershed exchanges with interior drainages rather than by dispersal of bull trout bearing group B haplotypes through the sea from the Chehalis/Fraser/lower Columbia area.

Diversity of bull trout mtDNA and conservation

The partitioning of bull trout mtDNA variation indicated that relatively little variation resides within individual sample sites, but that there is substantial variation among populations and among geographical regions. The variation within sample sites was typically less than 20% of the total from our AMOVA analyses, but sample sizes for many sites were low. For populations with at least 10 individuals sampled, however, the average haplotype diversity was only 0.21 and many populations were fixed for single haplotypes. By contrast, the observed bull trout haplotype diversity of 0.84 when pooled across localities is comparable (and higher than many) to values summarized for other freshwater fishes by Bernatchez & Wilson (1998). The low average haplotype diversity within populations and the variation among populations in molecular diversity, coupled with the high diversity 'species-wide' are consistent with the AMOVA results; mtDNA variation at the among-population level is substantial (cf. Leary *et al*. 1993; Williams *et al*. 1997). Further, average heterozygosity levels at allozyme and microsatellite loci assayed in bull trout populations (Leary *et al*. 1993; Spruell *et al*. 1999) are typically lower than reported for many other salmonid fishes (e.g. Allendorf & Leary 1988; Leary *et al*. 1993; Angers *et al*. 1995; Wenburg *et al*. 1998). The depauperate levels of variation within bull trout populations may result from repeated stochastic factors such as founder events, bottlenecks, and genetic drift in small populations, particularly during recolonization of northern, glaciated areas as the ice sheets retreated (Leary *et al*. 1993; Hewitt 1996; Merila *et al*. 1996). Such processes may be especially pronounced in bull trout which are top predators in aquatic ecosystems, typically have relatively small population sizes, and which exhibit strong site fidelity (Baxter 1997; Swanberg 1997). These same general life history features, however, would also be expected to promote a high level of population structure similar to that observed in our study and in allozyme and microsatellite assays (Leary *et al*. 1993; Spruell *et al*. 1999; A. Costello and E. B. Taylor, unpublished). The concentration of molecular variation among populations and geographical regions is often observed in freshwater fish species (e.g. Ward *et al*. 1994), but in bull trout, and perhaps char in general (Wilson *et al*. 1996; Angers & Bernatchez 1998), this pattern appears especially pronounced relative to many other salmonids (e.g. Allendorf & Leary 1988; Bernatchez & Osinov 1995). These observations emphasize that conservation of bull trout biodiversity, as measured by molecular assays, must focus on conservation of as many populations within as many different geographical regions as possible, because it is at these levels that the majority of molecular variation resides. In addition, although our focus was not on fine-scaled population structure, our results indicate a high degree of substructure within geographical regions (Table 3) and, consequently, substantial limits on gene flow among local populations. Because restricted gene flow favours divergence in different selective environments, it is probable that bull trout also exhibit interpopulation variation in quantitative traits. Molecular assays of diversity are, therefore, undoubtably conservative estimates of biodiversity in bull trout across populations and geographical regions.

Table 4 Distribution of restriction fragment length polymorphism (RFLP) mitochondrial DNA (mtDNA) composite haplotypes among bull trout sample populations. The numbers represent composite haplotypes defined in Table 2 and parenthetical number codes for populations correspond to those in Table 1 and Fig. 1

	Haplotype																				
Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Hotel (1)	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shilsky (2)	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Beaver (3)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crow (4)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesilinka (5)	5	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chowika (6)	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Osilinka (7)	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Burnt (8)	4	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Belcourt (9)	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dome (10)	6	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Small (11)	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UTorpy (12)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0
Chilko (13)	2	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grain (14)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NThompson (15)	3	0	3	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
Shuswap (16)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nahatlach (17)	3	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Dominion (18)	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
UAnderson (19)	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
UPitt (20)	0	0	0	0	0	0	0	0	0	4	15	0	0	0	0	0	0	0	0	0	0
Chillwack (21)	0	0	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0
Silverhope (22)	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Tahltan (23)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bulkley (24)	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nass (25)	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yakima (26)	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0	0	0	1	1	0	0
Duncan (27)	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salmo (28)	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0
Wigwam (29)	9	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Howell (30)	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anderson (31)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
Metolius (32)	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0
Clear Branch (33)	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Bear (34)	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
Jarbridge (36)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
Squamish (37)	0	0	0	0	0	0	0	0	0	0	9	1	0	0	0	0	0	0	0	0	0
Klinaklini (38)	7	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Puget Sound (39–41)	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
Skagit (42)	0	0	0	0	0	0	0	0	4	0	3	0	0	0	0	0	0	0	0	0	0
Elwha (43)	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0
Soleduck (44)	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
Long (45)	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
Belly (46)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Waterton (47)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	145	4	8	6	2	8	8	13	10	33	49	1	29	6	5	7	2	1	1	1	10

Note: population 35 was assayed only by sequence analysis.

Our data suggest that, at the coarsest level, conservation efforts for bull trout should recognize a distinction between coastal and interior assemblages of bull trout. Although our mtDNA data suggest that the divergence between coastal and interior lineages of bull trout is subtle (i.e. it is based more on the geographical distribution of closely related haplotypes rather than on deep evolutionary divisions), several other lines of evidence suggest a major subdivision of bull trout into coastal and interior lineages. First, a coastal–interior break in the distribution

of closely related haplotypes within the Columbia and Klamath systems is also apparent in the mtDNA data of Williams *et al*. (1997). Second, microsatellite data also exhibit a major coastal–interior split among bull trout populations in the US portion of the Columbia and in the Klamath River (P. Spruell, unpublished). Third, these molecular assays are mirrored by morphological analyses conducted by Haas (1988) and Cavender (1997) that also showed a coastal–interior division of bull trout. Finally, bull trout from several of the coastal lineage populations (Skagit (42), Squamish (37), and Pitt (20) rivers) are thought to be amphidromous (they make short forays into nearshore marine waters), a life history feature thought not to be expressed in interior populations even though many of the latter populations have unrestricted access to the sea (Cavender 1978; Haas & McPhail 1991; British Columbia Fish and Wildlife Branch, unpublished). Thus, the subdivision of bull trout into two major lineages, coastal and interior, in northwestern North America based on mtDNA is supported by concordance across a broad range of characteristics and supports the recognition of this primary division in conservation plans for the species (Moritz 1994; Waples 1995). Further, the presence of both groups of bull trout within the Columbia River is inconsistent with the view that only a single 'distinct population segment' characterizes Columbia River bull trout (US Department of Interior, Federal Register, July 1998, p. 31650). Rather, our data suggest that it would be appropriate to recognize the existence of at least two evolutionarily distinct units of bull trout within two of the major rivers within the species' range: the Columbia and Fraser rivers. Of course, the coastal–interior division represents only the broadest level of evolutionary divergence within bull trout and finer-scale distribution of population clusters within both interior and coastal lineages have been suggested to exist (Leary *et al*. 1993; Spruell *et al*. 1999).

Acknowledgements

Support for this research was provided by the Habitat Conservation Trust Fund and Forest Renewal B.C. administered by the B.C. Ministry of the Environment. We thank all the employees of B.C. Ministry of Environment regional offices who supervised the collection of many of our samples, Jim Reisling, Brian Klassen, and Al Stobbart for volunteer collection efforts, and P. Spruell and S. Young for providing some DNA samples. We appreciate the improvements to this paper stemming from comments by L. Bernatchez, S. Latham, and anonymous reviewers.

References

Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology*, **2**, 170–184.

Angers B, Bernatchez (1998) Combined use of SMM and non-SMM methods to infer structure and evolutionary history of closely-related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. *Molecular Biology and Evolution*, **15**, 143–159.

Angers B, Bernatchez L, Angers A, Desgroseillers L (1995) Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. *Journal of Fish Biology*, **47** (Suppl. A), 177–185.

Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.

Baxter JS (1997) Aspects of the reproductive ecology of bull trout (*Salvelinus confluentus*) in the Chowade River, British Columbia. MSc Thesis, University of British Columbia, Vancouver.

Baxter JS, Taylor EB, Devlin RH, Hagen J, McPhail JD (1997) Evidence for natural hybridization between Dolly Varden (*Salvelinus malma*) and bull trout (*S. confluentus*) in a northcentral British Columbia watershed. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 421–429.

Behnke RJ (1972) The systematics of salmonid fishes of recently glaciated lakes. *Journal of the Fisheries Research Board of Canada*, **29**, 639–671.

Behnke RJ (1980) A systematic review of the genus *Salvelinus*. In: (ed. Balon EK) *Charrs. Salmonid Fishes of the Genus* Salevlinus, pp. 441–481. Dr W. Junk Publishers, The Hague.

Bermingham E, Avise JC (1986) Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics*, **113**, 939–965.

Bernatchez L, Osinov A (1995) Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. *Molecular Ecology*, **4**, 285–297.

Bernatchez L, Wilson CC (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, **7**, 431–452.

Buckingham NM, Schreiner EG, Kaye TN, Burger JE, Tisch EL (1995) *Flora of the Olympic Peninsula*. Northwest Interpretive Association, Seattle.

Cavender TM (1978) Taxonomy and distribution of the bull trout, *Salvelinus confluentus* (Suckley), from the American Northwest. *California Fish and Game*, **64**, 139–174.

Cavender TM (1997) Morphological distinction of bull trout from the McCloud River system of northern California. In: (eds Mackay WC, Brewin MK, Monita M) *Friends of the Bull Trout Conference Proceedings*, pp. 271–281. Bull Trout Task Force (Alberta, Canada). Trout Unlimited Canada, Calgary.

Crane PA, Seeb LW, Seeb JE (1994) Genetic relationships among *Salvelinus* species inferred from allozyme data. *Canadian Journal of Fisheries and Aquatic Sciences*, **51** (Suppl. 1), 182–197.

Excoffier L, Smouse P, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Felsenstein J (1993) *PHYLIP: Phylogeny Inference Package, Version 3.5*. Department of Genetics, SK-50, University of Washington, Seattle.

Grewe PM, Billington N, Hebert PDN (1990) Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. *Canadian Journal of Fisheries and Aquatic Sciences*, **47**, 984–991.

Haas GR (1988) The systematics, zoogeography and evolution of Dolly Varden and bull trout in British Columbia. MSc Thesis, University of British Columbia, Vancouver.

Haas GR, McPhail JD (1991) The systematics, zoogeography and evolution of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 2191–2211.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hillis DM (1987) Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics*, **18**, 23–42.

Leary RF, Allendorf FW (1997) Genetic confirmation of sympatric bull trout and Dolly Varden in Western Washington. *Transactions of the American Fisheries Society*, **126**, 715–720.

Leary RF, Allendorf FW, Forbes SH (1993) Conservation genetics of bull trout in the Columbia and Klamath River drainages. *Conservation Biology*, **7**, 856–865.

Lindsey CC, McPhail JD (1986) Zoogeography of fishes of the Yukon and McKenzie Basins. In: (eds Hocutt CH, Wiley EO) *Zoogeography of North American Freshwater Fishes*, pp. 639–674. Wiley and Sons, New York.

Mackay WC, Brewin MK, Monita M (1997) *Friends of the Bull Trout Conference Proceedings*. Bull Trout Task Force (Alberta), c/o Trout Unlimited Canada, Calgary.

Martinson DG, Pisias NG, Hays JD, Imbrie J, Moore TC Jr, Shackleton NJ (1987) Age dating and the orbital theory of the ice ages: development of a high resolution 0–300,000-year chronostratigraphy. *Quaternary Research*, **27**, 1–29.

McElroy D, Moran P, Bermingham E, Kornfield I (1992) REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. *Journal of Heredity*, **83**, 157–158.

McPhail JD (1961) A systematic study of the *Salvelinus alpinus* species complex in North America. *Journal of the Fisheries Research Board of Canada*, **18**, 793–816.

McPhail JD (1967) Distribution of freshwater fishes in western Washington. *Northwest Science*, **41**, 1–11.

McPhail JD, Lindsey CC (1986) Zoogeography of the freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). In: (eds Hocutt CH, Wiley EO) *Zoogeography of North American Freshwater Fishes*, pp. 615–637. Wiley and Sons, New York.

McPhail JD, Taylor EB (1999) Morphological and genetic variation in northwestern longnose suckers, *Catostomus catostomus*—the Salish sucker problem. *Copeia*, 23–35.

Merila J, Bjorklund M, Baker AJ (1996) Genetic population structure and the gradual northward decline of genetic variability in the greenfinch (*Carduelis chloris*). *Evolution*, **50**, 2548–2557.

Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology*, **3**, 401–411.

Morton WM (1970) On the validity of all subspecific designations of North American *Salvelinus malma* (Walbaum). *Copeia*, **1970**, 581–587.

Nei M, Miller JC (1990) A simple method for estimating average number of nucleotide substitutions with and between populations from restriction data. *Genetics*, **125**, 873–879.

Nordeng H (1983) Solution to the 'char problem' based on Arctic char (*Salvelinus alpinus*) in Norway. *Canadian Journal of Fisheries and Aquatic Sciences*, **40**, 1372–1387.

O'Reilly P, Reimchen TE, Beech R, Strobeck C (1993) Mitochondrial DNA in Gasterosteus and Pleistocene glacial refugium on the Queen Charlotte Islands, British Columbia. *Evolution*, **47**, 678–684.

Ortí G, Bell MA, Reimchen TE, Meyer A (1994) Global survey of mitochondrial DNA sequences in the threespine stickleback: evidence for recent migrations. *Evolution*, **48**, 608–622.

Park LK, Brainward MA, Dightman DA, Winans GA (1993) Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (*Oncorhynchus keta*). *Molecular Marine Biology and Biotechnology*, **2**, 362–370.

Parkinson EA (1984) Genetic variation in populations of steelhead trout (*Salmo gairdneri*) in British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 1412–1420.

Pleyte K, Duncan SD, Phillips RB (1992) Evolutionary relationships of the genus *Salvelinus* inferred from the DNA sequences of the first internal transcribed spacer (ITS 1) of ribosomal DNA. *Molecular Phylogenetics and Evolution*, **1**, 223–230.

Redenbach Z, Taylor EB (1999) Zoogeographical implications of variation in mitochondrial DNA of Arctic grayling (*Thymallus arcticus*). *Molecular Ecology*, **8**, in press.

Rieman BE, Lee DC, Thurow RF (1997) Distribution, status and likely future trends of bull trout within the Columbia River and Klamath River basins. *North American Journal of Fisheries Management*, **17**, 1111–1125.

Sneath PHA, Sokal RR (1973) *Numerical Taxonomy*. W.H. Freeman & Sons, San Francisco.

Soltis DE, Gitzendanner MA, Strenge DD, Soltis PS (1997) Chloroplast DNA intraspecific phylogeography from the Pacific Northwest of North America. *Plant Systematics and Evolution*, **206**, 353–373.

Spruell P, Rieman BE, Knudsen KL, Utter FM, Allendorf FW (1999) Genetic population structure within streams: microsatellite analysis of bull trout populations. *Ecology of Freshwater Fish*, **8**, in press.

Swanberg TR (1997) Movements and habitat use by fluvial bull trout in the Blackfoot River, Montana. *Transactions of the American Fisheries Society*, **126**, 735–746.

Taylor EB (1995) Genetic variation at minisatellite DNA loci in North Pacific populations of steelhead and rainbow trout (*Oncorhynchus mykiss*). *Journal of Heredity*, **86**, 354–363.

Taylor EB, Bentzen P (1993) Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (genus *Osmerus*) in northeastern North America. *Evolution*, **47**, 813–822.

Taylor EB, Foote CJ, Wood CC (1996) Molecular genetic evidence for parallel life-history evolution within a Pacific salmon (sockeye salmon and kokanee, *Oncorhynchus nerka*). *Evolution*, **50**, 401–416.

Taylor EB, McPhail JD (1985a) Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 2020–2028.

Taylor EB, McPhail JD (1985b) Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus mykiss*. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 2029–2033.

Thompson CE, Taylor EB, McPhail JD (1997) Parallel evolution of lake–stream pairs of threespine sticklebacks (*Gasterosteus*) inferred from mitochondrial DNA. *Evolution*, **51**, 1955–1965.

Thorson RM (1980) Ice-sheet glaciation of the Puget lowland, Washington, during the Vashon state (late Pleistocene). *Quaternary Research*, **13**, 303–321.

Utter FM, Campton D, Grant WS, Milner G, Seeb J, Wishard L (1980) Population structure of indigenous salmonid species of the Pacific Northwest. In: (eds McNeil WJ, Himsworth D)

Salmonid Ecosystems of the North Pacific, pp. 285–304. Oregon State University Press, Corvallis.

Waples RS (1995) Evolutionary significant units and the conservation of biological diversity under the endangered species act. In: (ed. Neilsen JL) *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation*, pp. 8–27. American Fisheries Society Symposium 17, Bethesda.

Ward RD, Woodwark M, Skibinski DOF (1994) A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology*, **44**, 213–232.

Wehrhahn CF, Powell R (1987) Electrophoretic variation, regional differences, and gene flow in the coho salmon (*Oncorhynchus kisutch*) of southern British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **44**, 822–831.

Wenburg JK, Bentzen P, Foote CJ (1998) Microsatellite analysis of population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). *Molecular Ecology*, **7**, 733–749.

Williams RN, Evans RP, Shiozawa DK (1997) Mitochondrial DNA diversity patterns of bull trout in the upper Columbia River basin. In: (eds Mackay WC, Brewin MK, Monita M) *Friends of the Bull Trout Conference Proceedings*, pp. 283–297. Bull Trout Task Force (Alberta, Canada). Trout Unlimited Canada, Calgary.

Wilson CC, Hebert PDN, Reist JD, Dempson JB (1996) Phylogeography and postglacial dispersal of arctic charr *Salvelinus alpinus* in North America. *Molecular Ecology*, **5**, 187–197.

Wood CC, Riddell BE, Rutherford DT, Withler RE (1994) Biochemical genetic variation of sockeye salmon (*Oncorhynchus nerka*) in Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, **51** (Suppl. 1), 114–131.

The research on bull trout mtDNA variation represents a collaboration between the laboratory of E. B. Taylor and the British Columbia Ministry of the Environment (Fisheries Branch/Conservation Section) were S. Pollard is the conservation geneticist. E. B. Taylor's research group focuses on molecular ecology and population/conservation genetics of freshwater fishes. D. Louie was an undergraduate research assistant throughout the project.
